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INVESTIGATIONS IN FISH CONTROL

47. Preparation and Properties of Quinaldine Sulfate, an Improved Fish Anesthetic

48. Toxicity of Quinaldine Sulfate to Fish

49. The Efficacy of Quinaldine Sulfate as an Anesthetic for Freshwater Fish

50. Residue of Quinaldine in Ten Species of Fish Following Anesthesia with Quinaldine Sulfate

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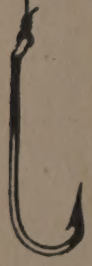

United States Department of the Interior
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P.O. Box 862
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United States Department of the Interior, Rogers C. B. Morton, *Secretary*
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FOREWORD

An anesthetic used on food and game fishes must bear a label registered by the U.S. Food and Drug Administration. The requirements for registration include data on the toxicity of the drug to exposed fish; on the efficacy as an anesthetic to selected fishes of several life stages in waters of different pH, hardness, and temperature; and on the residues of drug present in edible fish tissues following exposure. The following papers are concerned with results of registration-oriented research on toxicity, efficacy, and residues of quinaldine sulfate ($QdSO_4$) when employed as an anesthetic for selected species of coldwater and warmwater fish. The data will be used to support a petition for registration of the anesthetic.

Chemists at the Fish Control Laboratories developed quinaldine sulfate as an improved formulation of quinaldine, a coal tar derivative used extensively in the manufacture of dyes, pharmaceuticals, and fine organic chemicals. Quinaldine came into use as a fish anesthetic in 1958 and is preferred in some fish culture and fishery management operations because of its low order of toxicity to fish in long exposures. Although effective and economical, the 95-percent quinaldine used is an oily liquid that is insoluble in water and possesses a strong, disagreeable odor. In addition to these disadvantages, the 5 percent of other quinones present would have to be identified and studied in detail before an attempt was made to obtain registration of quinaldine as a fish anesthetic.

In contrast, quinaldine sulfate is a pure, crystalline formulation that is water soluble, has little odor, is easy to handle, and is effective as a fish anesthetic. Its purity would be an advantage in seeking registration. Thus, we chose to do registration-oriented research on quinaldine sulfate rather than on quinaldine.

Robert E. Lennon, Director
Fish Control Laboratories
June 2, 1972

47. Preparation and Properties of Quinaldine Sulfate, an Improved Fish Anesthetic

By John L. Allen and Joe B. Sills

United States Department of the Interior, Rogers C. B. Morton, *Secretary*
Nathaniel P. Reed, *Assistant Secretary for Fish and Wildlife and Parks,*
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PREPARATION AND PROPERTIES OF QUINALDINE SULFATE, AN IMPROVED FISH ANESTHETIC

By John L. Allen and Joe B. Sills
Fish Control Laboratories, La Crosse, Wisconsin
and Warm Springs, Georgia

ABSTRACT.--The preparation of quinaldine sulfate ($QdSO_4$) from practical grade quinaldine and sulfuric acid yields a crystalline product which is water soluble and easily purified by recrystallization. The product conforms with the empirical formula $C_{10}H_{11}NSO_4$ and assays 99.4 percent pure by titration. The crystalline material has little odor and is water soluble; thus, it is less objectionable and much easier to handle than quinaldine for anesthetizing fish.

INTRODUCTION

Quinaldine (2-methylquinoline) was suggested as an anesthetic for fish by Muench (1958) and has been used safely and effectively to facilitate general handling of fishes over a wide variety of conditions. This chemical presumably enters a fish's body by absorption across the gill membranes, but its specific physiological mode of action has not been determined. At a concentration of 10 mg/l, quinaldine has been shown by Clark and Granath (1968) to depress the frequency of electrical discharge of the gymnotid eel (Sternarchus albifrons). Trams and Brown (1970) using brain homogenates from nurse sharks (Ginglymostoma cirratum) and cow-nosed rays (Rhinoptera bonasus) have shown that 10^{-3} M quinaldine produced a 50-percent inhibition of acetylcholinesterase and butyrycholinesterase. However, Trams and Brown do not think this is the mode of action of quinaldine.

Practical grade quinaldine is an economical and effective tool; however, it is an oily liquid not readily soluble in water, and has a strong, disagreeable odor. Jodlbauer and Salvendi (1905) noted that quinoline and the sulfuric acid salt of quinoline both exhibited anesthetic properties with fish. Sills and Harman (1970) showed quinaldine sulfate possesses the same desirable anesthetic properties as quinaldine. The salt is a crystalline form of the anesthetic with none of the unfavorable characteristics of the liquid, and is a product of high purity. The objectives of this paper are to present the preparation of the quinaldine salt and its chemical characterization as an improved formulation for use as an anesthetic by fishery workers.

METHODS AND MATERIALS

Since commercial sources of quinaldine sulfate could not be located, the quinaldine sulfate was prepared from Eastman Kodak, 95-percent quinaldine. Initially, two small batches of quinaldine sulfate were prepared by reacting 20 ml of quinaldine with 7.0 ml of concentrated sulfuric acid in the presence of a hydrocarbon solvent. Later, four batches of quinaldine sulfate (2-methylquinoline sulfate) were prepared using the following batch formula:

quinaldine, 500 grams or 3.49 moles
sulfuric acid, 190 ml or 3.42 moles
iso-octane, 500 ml.

The quinaldine and iso-octane were placed in a 4,000-ml reaction flask and were mixed thoroughly with a mechanical stirrer. The reaction mixture was placed in an ice bath, and concentrated sulfuric acid was added slowly while the

mixture was stirred vigorously. The rate of sulfuric acid addition was adjusted to keep the temperature of the mixture below the boiling point of iso-octane (99.3° C.). The resultant mixture was filtered using vacuum, and the filter cake was washed with approximately 250 ml of benzene. The product was recrystallized from methanol. The recrystallization yielded approximately 566 grams of a bright red crystalline material (68.5-percent yield).

RESULTS

The quinaldine sulfate was tested for purity and for structural changes as a result of the reaction of sulfuric acid with quinaldine. The recrystallized quinaldine sulfate was soluble in water and alcohol and insoluble in benzene and ether (table 1.). The melting range of the material was 211°-214° C. Stecher et al. (1968) list the melting range of quinaldine sulfate as 211°-213° C. When titrated to a phenolphthalein end point with 0.1 N sodium hydroxide, the product was determined to be 99.4 percent $C_{10}H_{11}NSO_4$. The infrared spectra of practical grade quinaldine and the free base of quinaldine sulfate were determined in carbon tetrachloride (from 4,000 to 1,300 cm^{-1}) and in carbon disulfide (from 1,300 to 400 cm^{-1}). The spectrum of free base of quinaldine sulfate shows all the absorption bands present in the spectrum of practical grade quinaldine (fig. 1). The infrared spectrum of quinaldine sulfate in a KBr pellet also contains bands at 1,000 and 1,170 cm^{-1} indicating presence of sulfate and a broad band at 2,700 cm^{-1} which indicates an amine salt. The ultraviolet spectrum of the product in 0.1 N H_2SO_4 gives absorption maxima at 236 and 317 nm (fig. 2).

TABLE 1.--The solubility of quinaldine sulfate in seven different solvents at 20° C.

Solvent	Solubility (g/100 ml)
water	104.05
methyl alcohol	7.44
ethyl alcohol	2.27
acetone	0.08
ethyl ether	[1] i
benzene	i
hexane	i

[1] Insoluble = less than 0.01 grams dissolves in 100 ml of solvent.

An aqueous solution of quinaldine sulfate was made alkaline and extracted with hexane to give a solution of the free base of quinaldine in hexane. The resulting hexane solution was injected into a gas chromatograph equipped with a 180 cm x 4 mm column packed with 5-percent carbowax 20 M on gas chrom Q 80/100 mesh at 130° C. and a flow rate of 60 ml/minute. A single peak with the same retention time as quinaldine eluted after the solvent peak.

The product gives a positive test for sulfate using the USP XVII (1965) test for sulfates.

Two batches of quinaldine sulfate prepared by the above method were subjected to elemental analysis[1] with the following results.

	%C	%H	%N	%S	%O
Calculated	49.78	4.60	5.81	13.29	26.53
Sample 1	49.40	4.59	5.89	13.15	26.20
Sample 2	49.48	4.57	5.83	13.39	26.79

The elemental analysis indicates an empirical formula of $C_{10}H_{11}NSO_4$, which is the empirical

[1] The elemental analysis was done by Galbraith Laboratories, Inc., Knoxville, Tennessee.

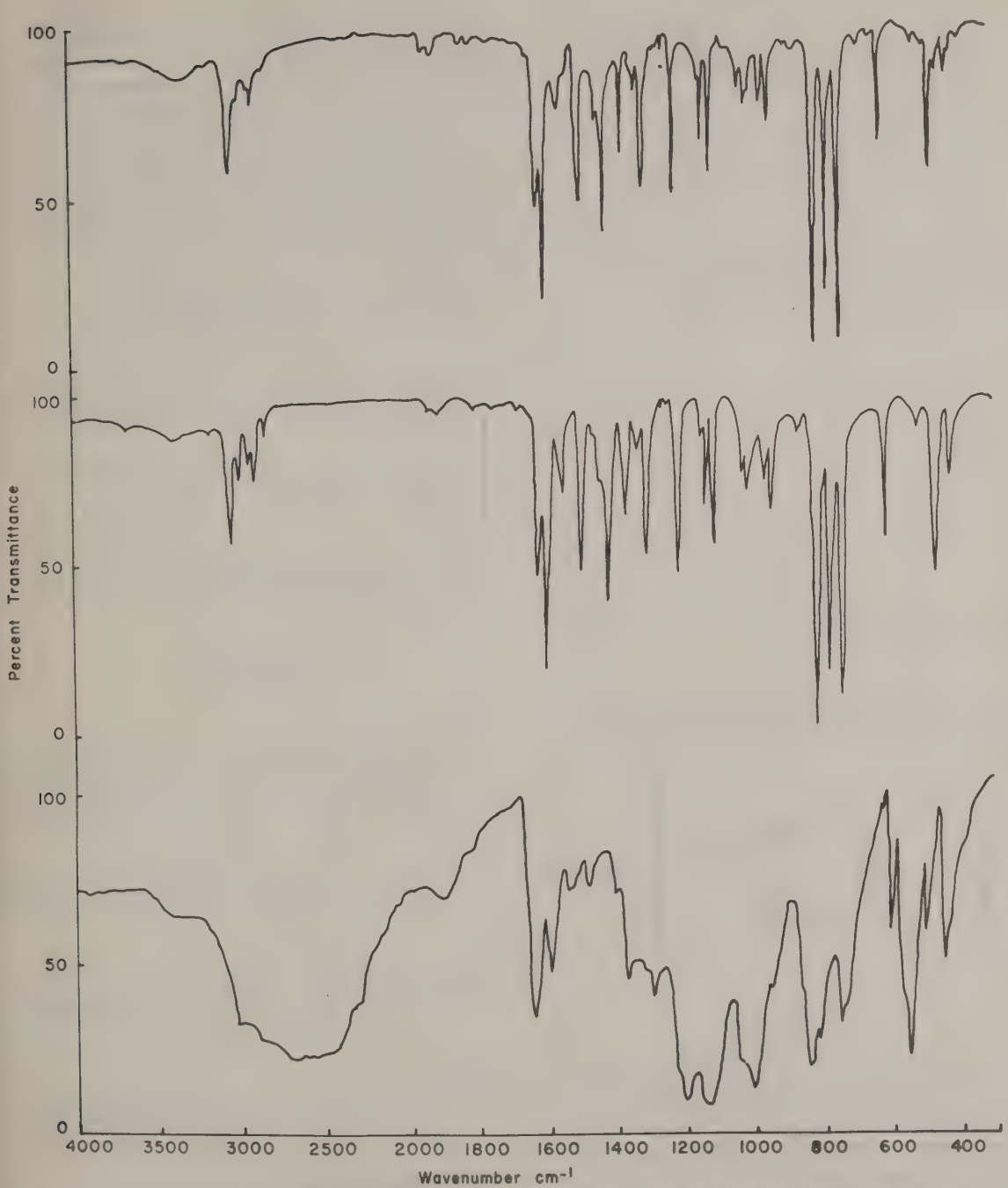

Figure 1.--Infrared spectra of Eastman Kodak practical grade quinaldine (top), and the free base of quinaldine sulfate middle) in carbon tetrachloride and carbon disulfide, and quinaldine sulfate (bottom) in a KBr pellet.

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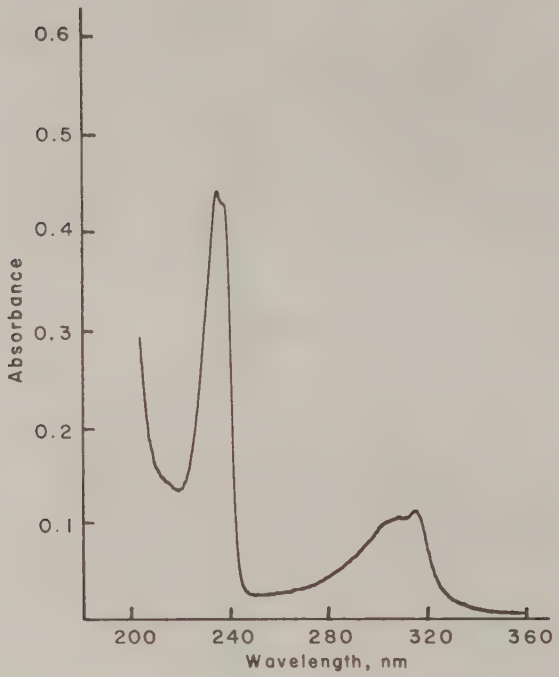
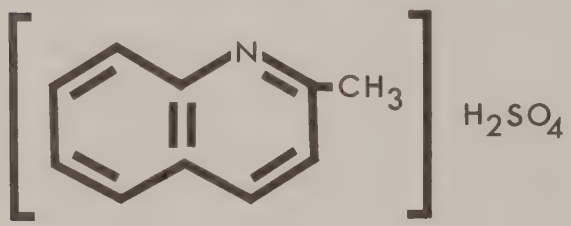


Figure 2.--Ultraviolet spectrum of quinaldine sulfate in 0.1 N sulfuric acid.

formula of quinaldine sulfate given by Stecher et al. (1968). The product can be represented by the semi-structural formula given below.



DISCUSSION

The quinaldine sulfate prepared during this study has been shown to be a product of high purity. The reaction of sulfuric acid with quinaldine using the conditions of preparation we used in this investigation did not alter the structure of the quinaldine molecule. The sulfate salt of the nitrogenous base (quinaldine) was recovered in good yield (68.5 percent).

Schoettger and Julin (1969) indicated that quinaldine is inactive as an anesthetic for fish at pH 6 or below. Sills and Allen (1971) correlated the efficacy of quinaldine at a given pH with the ionization constant of the molecule. They indicated the uptake of the chemical by fish is dependent on the amount of the quinaldine present in the water in the lipophilic, free base form. Quinaldine sulfate is an acid salt and will depress the pH of anesthetic solutions, particularly in soft water. The depression of pH can be overcome by buffering the anesthetic solutions.

CONCLUSIONS

1. Recrystallization of quinaldine sulfate prepared by the reaction of quinaldine with sulfuric acid gives a product of high purity.

2. Quinaldine sulfate is a water soluble crystalline material with little odor.

3. Since this salt is also an effective anesthetic, it is a more useful tool for fishery work than practical grade quinaldine.

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48. Toxicity of Quinaldine Sulfate to Fish

By Leif L. Marking and Verdel K. Dawson

United States Department of the Interior, Rogers C. B. Morton, *Secretary*
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TOXICITY OF QUINALDINE SULFATE TO FISH

By Leif L. Marking and Verdel K. Dawson
Fish Control Laboratories, La Crosse, Wisconsin

ABSTRACT.--The acute toxicities of the candidate fish anesthetic, quinaldine sulfate ($QdSO_4$), were determined against selected species of cold-water and warmwater fishes. The LC50's (concentrations producing 50-percent mortality) were derived for 3-, 6-, 24-, and 96-hour exposures in bioassays with different temperatures, hardnesses, and pH's. The 96-hour LC50's of $QdSO_4$ ranged from 6.8 mg/l for largemouth bass to 72.5 mg/l for carp. In very soft water $QdSO_4$ solutions are acidic and considerably less toxic than in harder water. This lack of activity is attributed to a decrease in the pH of the test solution which thereby decreases the concentration of the active, un-ionized form of the molecule.

INTRODUCTION

Quinaldine sulfate ($QdSO_4$) is a candidate fish anesthetic (Allen and Sills, 1973). An early step in its development and possible registration as a fishery tool is definition of its toxicity to selected fishes in standard bioassays in waters with different hardnesses, pH's, and temperatures (Lennon, 1967). Observations also were made on the influence of quinaldine sulfate on bioassay media and on degradation of the compound.

METHODS AND MATERIALS

Static bioassays of $QdSO_4$ were conducted with 3- to 14-cm fish in 15-liter glass jars according to the methods of Lennon and Walker (1964).

Larger fish were exposed to the anesthetic in 45-liter polyethylene tanks. The fish were obtained from fish hatcheries, maintained under a fish culturist's care (Hunn, Schoettger, and Whealdon, 1968), acclimated according to standard bioassay procedures, and incinerated after death. Ten fish were exposed to each concentration of the anesthetic, and mortalities were recorded periodically during the first day and daily thereafter during the 96-hour tests.

Variations in test water were produced by adding different amounts of reconstituting salts to deionized water (table 1). The pH in various tests was adjusted and maintained with chemical buffers (table 2). Temperatures of 7°, 12°, and 17° C. were controlled by water baths.

TABLE 1.--Quantities of salts and characteristics of reconstituted waters

Water type	Salts added (mg/l)				pH range	Total	
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl		Hardness[2]	Alkalinity[2]
very soft	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
soft[1]	48	30	30	2.0	7.2-7.6	40-48	30-35
hard	192	120	120	8.0	7.6-8.0	160-180	110-120
very hard	384	240	240	16	8.0-8.4	280-320	225-245

[1] Standard reconstituted water used in routine bioassays.
[2] As mg/l $CaCO_3$.

TABLE 2.--Buffer chemicals for adjusting the pH of standard reconstituted water at 12° C.

pH	Ml of solutions for 15 liters of water		
	1N NaOH	1M KH_2PO_4	0.5M H_3BO_3
6.0	1.3	80.0	--
6.5	10.0	30.0	--
7.0	19.0	30.0	--
7.5	--	--	--
8.0	19.0	20.0	--
8.5	12.0	11.5	--
9.0	8.8	--	30.0
9.5	11.0	--	20.0
10.0	16.0	--	18.0

The anesthetic was dissolved in water, and aliquots were added to the bioassays to obtain the desired concentrations.

The mortality data were analyzed according to the method of Litchfield and Wilcoxon (1949) to determine LC50's, variations, slope functions, and 95-percent confidence intervals.

RESULTS

Effects of $QdSO_4$ on test solutions

Stock solutions of $QdSO_4$ are acidic and influence the pH of bioassay water, especially softer waters. Stock solutions containing 75 grams of $QdSO_4$ in a liter of deionized water have a pH of 1.5. Since different amounts of $QdSO_4$ are added to bioassays to obtain data on the survival and mortality of fish, the chemical properties of the test solutions must be considered. Concentrations ranging from 0 to 80 mg/l of $QdSO_4$ were prepared in waters of various hardnesses, and the pH's were recorded (table 3). The pH in very soft water drops from 6.55 to 3.86 with the addition of 80 mg/l of $QdSO_4$. In harder waters which have a higher buffer capacity, the pH is more stable and decreases only one pH unit. These factors must be considered when assessing the toxicity of $QdSO_4$ to fish.

TABLE 3.--The influence of quinaldine sulfate on the pH of hard and soft water test solutions

Quinaldine sulfate (mg/l)	pH of different test solutions			
	Very soft	Soft	Hard	Very hard
0	6.55	7.10	7.78	8.00
5	6.29	6.92	7.57	7.82
20	5.65	6.61	7.30	7.55
40	5.40	6.22	7.00	7.39
60	4.02	6.04	6.87	7.19
80	3.86	5.67	6.61	7.01

Species and sizes of fish

Among the coldwater species, lake trout are the most sensitive to $QdSO_4$ at all exposure periods (LC50 = 26.3 mg/l at 3 hours and 15.5 mg/l at 96 hours) (table 4). Brook trout are the most resistant in 3-hour exposures (LC50 = 83.0 mg/l), while coho salmon are the most resistant at 96 hours (LC50 = 32.8 and 42.0 mg/l).

Among the warmwater species, largemouth bass are the most sensitive to $QdSO_4$ (96-hour LC50 = 6.80 mg/l). The most highly resistant species, carp and black bullhead, have 96-hour LC50's of 72.5 and 72.0 mg/l, respectively (table 4). In 96-hour exposures, largemouth bass and walleye are more sensitive than lake trout, and green sunfish are more sensitive than rainbow trout or brown trout.

Effects of temperature and water hardness

Rainbow trout are more susceptible to the toxic properties of $QdSO_4$ in cold water than in warm water in exposures of 1 to 6 hours (table 5). This trend reverses in 96-hour exposures, however, and the anesthetic is more toxic at 17° C. than at 7° or 12° C. At 24 hours the LC50 is 23.8 mg/l for 7° and 17° C., but at 12° C. the trout are more resistant (LC50 = 37.0 mg/l). Warmer temperatures cause greater variations in toxicity. At 17° C. the LC50 decreases from 47.0 at 1 hour to 12.8 mg/l at 96 hours, while at 7° C. the LC50 decreases from 28.9 at 1 hour to 23.8 mg/l at 96 hours. The most significant

TABLE 4.--Toxicity of quinaldine sulfate to fish in standard reconstituted water at 12° C.

Species	Average length (cm)	Average weight (grams)	LC50 and 95-percent confidence interval (mg/l) at			
			3 hours	6 hours	24 hours	96 hours
Coho salmon (Oncorhynchus kisutch)	4.1	0.7	80.0 55.1-116	80.0 55.1-116	47.5 41.9-53.9	32.8 30.0-35.9
"	11.4	17.0	---	70.0 52.1-94.0	45.0 42.1-48.1	42.0 37.6-46.9
Rainbow trout (Salmo gairdneri)	4.1	0.6	41.1 38.0-44.5	40.0 36.1-44.3	37.0 32.5-42.2	31.8 28.7-35.2
Brown trout (Salmo trutta)	3.8	0.6	55.0 47.0-64.4	---	32.7 28.5-37.5	28.3 25.7-31.2
Brook trout (Salvelinus fontinalis)	4.8	1.2	83.0 55.5-124	59.0 46.6-74.7	27.2 23.7-31.3	22.2 19.5-25.2
"	13.7	31.0	39.0 36.2-42.1	36.0 30.8-42.1	20.0 16.3-24.5	20.0 16.3-24.5
Lake trout (Salvelinus namaycush)	4.1	0.5	26.3 23.1-30.0	23.4 20.8-26.3	16.3 14.3-18.6	15.5 13.7-17.5
Carp (Cyprinus carpio)	4.6	1.4	85.4 73.2-99.7	80.0 64.9-98.6	74.6 59.3-93.8	72.5 59.7-88.1
Black bullhead (Ictalurus melas)	4.6	1.2	---	---	102 66.7-156	72.0 57.6-90.0
Channel catfish (Ictalurus punctatus)	5.6	1.5	60.0 53.7-67.1	49.4 43.3-56.4	39.1 34.0-45.0	32.9 27.4-39.5
Green sunfish (Lepomis cyanellus)	4.3	1.5	---	---	23.5 21.8-25.4	23.5 21.8-25.4
Bluegill (Lepomis macrochirus)	4.3	1.5	44.1 39.4-49.4	42.3 37.4-47.2	36.8 32.5-44.7	32.0 27.3-37.5
Largemouth bass (Micropterus salmoides)	4.6	1.4	20.8 18.3-23.5	19.4 17.1-22.0	16.0 9.64-26.7	6.80 3.71-13.6
Walleye (Stizostedion vitreum)	5.3	1.3	17.9 15.9-20.1	17.9 15.9-20.1	17.2 15.4-19.2	15.0 12.1-18.5

increase in toxicity is at 17° C. in 24 and 96 hours.

Rainbow trout are least susceptible to the toxic effects of $QdSO_4$ in very soft water (12 mg/l of total hardness) (table 5). Extremely high concentrations of the anesthetic are necessary in very soft water to kill the trout in 1- to 6-hour exposures. None of the trout died when exposed for 1 hour to 140 mg/l of the drug. The decreased activity of $QdSO_4$ in very soft water can be attributed to a decrease in pH as indicated in table 3. At 140 mg/l of $QdSO_4$, the pH of the test solution dropped to 3.35, which is below the pKa value of 5.42 (Knight et al., 1955; Sober, 1968). The equilibrium, therefore, is shifted in favor of the ionized form which is relatively unavailable to the fish (Sills and Allen, 1971). The extreme pH condition perhaps contributed to the mortality by stressing the fish.

In soft water (44 mg/l of total hardness), $QdSO_4$ is considerably more toxic than in very soft water, and LC50's range from 46.1 mg/l at 1 hour to 31.8 mg/l at 96 hours (table 5). In hard water (170 mg/l of total hardness) and in very hard water (300 mg/l of total hardness), the toxicity of $QdSO_4$ to rainbow trout is insignificantly different at each exposure (table 5). Also, the LC50's change very little in 1- to 96-hour exposures at each water hardness, but the values clearly show that $QdSO_4$ is consistently more toxic to trout in harder than in softer water at 12° C.

Effects of buffered and aged solutions of $QdSO_4$

Selected pH's ranging from 6 to 10 were produced in soft water (44 mg/l of total hardness) with the buffering agents listed in table 2. These

TABLE 5.--Toxicity of quinaldine sulfate to rainbow trout at different temperatures and water hardness

Water hardness	Temp. (°C.)	LC50 and 95-percent confidence interval (in mg/l) at				
		1 hour	3 hours	6 hours	24 hours	96 hours
soft[1]	7	28.9 24.3-34.4	28.5 24.7-32.1	25.8 22.6-29.4	23.8 20.3-27.9	23.8 20.3-27.9
soft[1]	12	46.1 41.4-51.4	41.1 38.0-44.5	40.0 36.1-44.3	37.0 32.5-42.2	31.8 28.7-35.2
soft[1]	17	47.0 39.5-55.9	46.0 40.6-52.1	42.5 35.0-51.7	23.8 19.2-29.5	12.8 11.1-14.9
very soft	12	---	133 116-153	90.5 83.2-98.4	65.5 62.2-69.0	50.5 46.0-55.5
hard	12	30.0 27.3-32.9	29.6 28.3-31.0	29.6 28.3-31.0	23.5 21.3-25.9	22.9 21.9-24.9
very hard	12	28.9 26.0-32.1	28.2 24.8-32.0	28.2 24.8-32.0	25.0 21.9-28.5	23.0 20.1-26.3

[1] Standard reconstituted water used in routine bioassays.

data were analyzed at 24 hours because $QdSO_4$ does not kill the trout in shorter exposures in the acid waters tested, and buffer chemicals complicate the toxicity of chemicals to rainbow trout at very high and very low pH levels in longer exposures (Marking, 1969). Despite the buffering capacity of these solutions, the pH decreased proportionately with concentrations, and the changes were greatest at pH 6. For instance, 20.0 mg/l of $QdSO_4$ in water buffered to pH 6 decreased the pH to 5.85, whereas 400 mg/l of the anesthetic in that water decreased the pH to 3.30.

The 400-mg/l concentration did not kill any rainbow trout at 6 hours, but they all died within 24 hours. Again, we suspect that the fish were stressed by the low pH. The $QdSO_4$ is more toxic in near neutral or basic water than in acid water (table 6), and LC50's range from 20.7 to 62.0 mg/l at pH 10 and 6, respectively. The most

TABLE 6.--Toxicity of quinaldine sulfate to rainbow trout in pH buffered water, in pH readjusted buffered water, and in aged (1 week) pH readjusted buffered water at 12° C.

pH (original)	24-hour LC50 and 95-percent confidence interval in mg/l in		
	pH buffered tests (fresh)	pH readjusted tests[1] (fresh)	pH readjusted tests[1] (aged)
6.0	62.0 54.1-71.1	47.1 40.7-54.5	40.0 33.0-48.6
[2] 7.4	37.0 32.5-42.2	26.3 23.5-29.4	28.2 25.6-31.1
8.0	23.2 20.0-27.0	24.4 21.0-28.3	23.8 20.6-27.6
9.0	25.0 22.7-27.5	25.7 22.4-29.4	23.8 21.6-26.2
10.0	20.7 18.5-23.2	21.1 18.0-24.8	15.9 13.5-18.8

[1] Readjusted to desired pH at all concentrations of quinaldine sulfate.
[2] Standard reconstituted water.

significant decrease in activity is from pH 7.4 to pH 6.

In tests in alkaline waters of pH 8, 9, and 10, where the pH was readjusted to original buffered pH's at all concentrations of $QdSO_4$, the toxicity was insignificantly different than unadjusted tests (table 6). The toxicity of $QdSO_4$ increased in near neutral and acid waters when the pH was readjusted to 7.4 and 6.0, and LC50's are 26.3 and 47.1 mg/l, respectively. Again, $QdSO_4$ is more toxic in high pH waters.

Concurrent with the fresh, pH readjusted tests, similar concentrations of $QdSO_4$ were pH readjusted and aged for 1 week prior to introducing rainbow trout into the bioassays. The aged, pH readjusted solutions of $QdSO_4$ remain as active as fresh, pH readjusted solutions at the pH's tested (table 6). In fact, the aged, pH readjusted solutions are significantly more toxic at pH 10.

DISCUSSION

Quinaldine sulfate is consistently less toxic to fish than quinaldine (Marking, 1969). The 96-hour LC50's for $QdSO_4$ when tested against brook trout and channel catfish were 20.0 and 32.9 mg/l, respectively. The 96-hour LC50's for quinaldine were 12.0 mg/l for brook trout and 19.9 mg/l for channel catfish. However, when the concentrations are computed on the basis of active ingredients, the formulations are approximately equal in activity.

The activity of $QdSO_4$ is similar at various temperatures to that of quinaldine (Marking, 1969). Both compounds are more toxic at colder temperatures in 1- to 6-hour exposures, but the trend begins to change at 24 hours and reverses at 96 hours for the extreme temperatures. These data suggest that exposures at warmer temperatures are perhaps safer (less toxic), providing the exposures are short. In contrast, MS-222 (a fish anesthetic) is more toxic to fish at warmer temperatures (Marking, 1967).

Tests in very soft and acid water indicate that the activity of $QdSO_4$ is decreased considerably in these waters. In agreement, Schoettger and Julin (1969) and Sills and Allen (1971) report that quinaldine loses its effectiveness in solutions having pH values less than 6. Since rainbow trout are generally intolerant to high or low pH's, we suspect that the low pH is more responsible for mortality than the $QdSO_4$ in this water.

CONCLUSIONS

1. $QdSO_4$ is toxic to fish, and the 96-hour LC50's for 12 species in standard reconstituted water range from 6.80 to 72.5 mg/l Carp are most resistant, while lake trout are most sensitive.

2. $QdSO_4$ is more toxic to rainbow trout in cold water than in warm water in 1- to 6-hour exposures, but the trend reverses at 96 hours.

3. The anesthetic is more toxic in hard than in soft water, but the increased pH in hard water perhaps contributes to the greater toxicity.

4. Higher concentrations of $QdSO_4$ decrease the pH of buffered and nonbuffered test solutions significantly, thereby decreasing the concentration of the un-ionized quinaldine. We suspect that the low pH stressed the fish and contributed to the mortality. The anesthetic is consistently toxic in buffered solutions of pH 8, 9, and 10, but the drug is considerably less toxic at pH 6.

5. The anesthetic in reconstituted water remains as toxic in solutions aged for 1 week as in fresh solutions.

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49. The Efficacy of Quinaldine Sulfate as an Anesthetic for Freshwater Fish

By Philip A. Gilderhus, Bernard L. Berger,
Joe B. Sills, and Paul D. Harman

United States Department of the Interior, Rogers C. B. Morton, *Secretary*
Nathaniel P. Reed, *Assistant Secretary for Fish and Wildlife and Parks,*
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife, Spencer H. Smith, *Director*
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THE EFFICACY OF QUINALDINE SULFATE AS AN ANESTHETIC FOR FRESHWATER FISH

By Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman

ABSTRACT.--Quinaldine sulfate ($QdSO_4$), an improved formulation of the fish anesthetic quinaldine, was tested for its efficacy on 15 species of freshwater fish. The new crystalline formulation is water soluble and thus easier to use than is practical grade quinaldine. $QdSO_4$ anesthetizes most salmonids at 25 mg/l in less than 4 minutes and the fish recover in 1 to 13 minutes in fresh water. The effective concentrations for several warmwater species varied from 15 mg/l for bluegills to 60 mg/l for channel catfish. Fish were held for up to 60 minutes in effective concentrations without suffering mortalities. The efficacy of the anesthetic was little affected by water temperature, but the compound lowers the pH of some soft waters to below 6, the point at which it becomes ineffective as an anesthetic. All fish retained some reflex action and thus, some large fish were difficult to handle.

INTRODUCTION

Quinaldine (2-methylquinoline) has been used to anesthetize fish for spawning, marking, measuring, and long-distance transport of fish. Schoettger and Julin (1969) demonstrated that practical grade quinaldine anesthetizes several species of coldwater and warmwater fish to a handleable condition at 15 to 70 mg/l in 2 minutes or less. Fish were held for up to 6 hours in effective concentrations and recovered in less than 1 hour after their return to fresh water.

Practical grade quinaldine (liquid, 95 percent), however, is insoluble in water and has a disagreeable odor. To overcome these disadvantages, Allen and Sills (1973) synthesized quinaldine sulfate ($QdSO_4$), a crystalline salt of quinaldine, which is water soluble and has little or no odor. Marking and Dawson (1973) have investigated the toxicity of quinaldine sulfate and found the material generally of comparable or lower toxicity than practical grade quinaldine (Marking, 1969) on an active ingredient basis using fish of comparable size at the same water temperature.

The efficacy of $QdSO_4$ was tested on freshwater fish to provide guidelines for use by fishery workers and to provide the proof of efficacy required by the regulatory agencies for registration.

METHODS AND MATERIALS

The quinaldine sulfate (59.3 percent quinaldine) used in these tests was formulated at the Southeastern Fish Control Laboratory at Warm Springs, Georgia. This compound is an experimental drug and is not registered for use as an anesthetic for fish. Aliquots of anesthetic were weighed individually for each concentration or test vessel.

Depending on the availability of fish, the anesthetic was tested on some species at the Fish Control Laboratory, La Crosse, Wisconsin, on some at the Warm Springs laboratory, and on some at both laboratories. Field

tests were conducted at several fish hatcheries where fish were anesthetized during spawning operations.

Tests using fingerling-size fish were conducted in 15-liter glass jars. Larger fish were exposed to the anesthetic in the laboratory and field in 45- and 100-liter polyethylene tanks. Water temperatures were adjusted and maintained by placing the test vessel in a water bath of the appropriate temperature.

The species of fish exposed to the anesthetic were coho salmon (Oncorhynchus kisutch), rainbow trout (Salmo qairdneri), brown trout (Salmo trutta), brook trout (Salvelinus fontinalis), lake trout (Salvelinus namaycush), northern pike (Esox lucius), goldfish (Carassius auratus), carp (Cyprinus carpio), white amur (Ctenopharyngodon idella), white sucker (Catostomus commersoni), black bullhead (Ictalurus melas), channel catfish (Ictalurus punctatus), bluegill (Lepomis macrochirus), largemouth bass (Micropterus salmoides), and walleye (Stizostedion vitreum).

The fish for laboratory tests were obtained from Federal and State fish hatcheries or, in the case of small coho salmon and rainbow trout, hatched and reared at the La Crosse laboratory. All fish tested in the laboratories were maintained as described by Hunn et al. (1968). The fish were acclimated to the test water and temperature for 16 to 24 hours before their exposure to the anesthetic.

The laboratory tests at La Crosse were conducted in well water and soft, reconstituted water. Those at Warm Springs were conducted in limed spring water (table 1). The field tests were conducted in the water supplies of the respective fish hatcheries (table 2).

TABLE 1.--Characteristics of waters used in laboratory tests of quinaldine sulfate as an anesthetic for fish

Source	Type	pH	Total hardness mg/l	Total alkalinity mg/l
Well--La Crosse	hard	7.6-7.9	238-258	218-230
Reconstituted--La Crosse	soft	7.2-7.6	40-48	30-35
Spring--Warm Springs	soft	6.8-7.0	20	---

TABLE 2.--Characteristics of hatchery water supplies used in tests of $QdSO_4$ to anesthetize adult fish

Location	Species tested	Water temp. (°C.)	pH	Total alkalinity (mg/l)	Total hardness (mg/l)
Platte River SFH[1] Michigan	coho salmon	6	7.8	150	168
Manchester NFH[2] Iowa	rainbow trout	9	7.5	172	215
Manchester NFH Iowa	brown trout	8	---	---	---
Osceola SFH Wisconsin	brook trout	9	8.1	171	208
Crystal Springs SFH Minnesota	lake trout	8	7.5	257	280
Lansing SFH Iowa	northern pike	5	9.2	141	144
Lansing SFH Iowa	walleye	10	9.2	133	164

[1] State fish hatchery.
[2] National fish hatchery.

The temperatures of test solutions were 7°, 12°, 17°, 22°, and 27° C. at La Crosse and 19° C. at Warm Springs. Field tests were conducted at the existing temperatures of the hatchery water supplies.

Schoettger and Julin (1967) defined loss of equilibrium, Stage 2, as the stage of anesthesia at which locomotion ceases, opercular rate slows, but reflex response to pressure on the caudal fin or peduncle is retained. In their tests with quinaldine, Schoettger and Julin (1969) found that the depth of anesthesia in fish rarely progresses beyond loss of equilibrium, Stage 2. Therefore, the majority of our tests were designed to define the most economical concentrations which would anesthetize 100 percent of the individual fish exposed, to loss of equilibrium, Stage 2, in 4 minutes or less. These criteria appear to satisfy the requirements for most of the field uses of fish anesthetics.

RESULTS

Reactions of fish

The reactions of fish to quinaldine sulfate were similar to their reactions to practical grade quinaldine as described by Schoettger and Julin (1969). Initially the fish show a short period of increased activity, but the anesthetization progresses rapidly to loss of equilibrium, Stage 2, and rarely progresses beyond that stage.

The fish usually retained reflex action which could be triggered by tactile stimuli. The reflex action in large adult fish sometimes made them difficult to handle without injury to the fish. Northern pike were most likely to display this action and salmonids the least.

Efficacy of the anesthetic

Twenty to 30 mg/l of $QdSO_4$ anesthetized the five species of salmonids to loss of equilibrium, Stage 2, in approximately 4 minutes or less (table 3). Twenty-five mg/l of $QdSO_4$ (15 mg/l of quinaldine) were effective on most species and sizes of salmonids, comparing favorably with the 15 to 16 mg/l of quinaldine found to be effective by Schoettger and Julin (1969). All of the salmonids in our tests recovered in fresh water within 1 to 13 minutes compared to 3 to 21 minutes for salmonids in similar exposures to quinaldine as stated by Schoettger and Julin (1969). Fish were retained up to 60 minutes in loss of equilibrium, Stage 2, without any apparent harm.

TABLE 3.--Efficacy of quinaldine sulfate as an anesthetic for salmonids in laboratory tests

Species	Mean weight (grams)	Type of water	Temp. (°C.)	Concentration (mg/l)	Number of fish	Exposure time (min.)	Time in minutes to	
							Loss of equilibrium Stage 2	Recovery
Coho salmon	12	hard	12	20	15	15-60	0.9-1.3	1.0-2.8
Rainbow trout	450	hard	7	25	15	15	1.0-1.8	6.0-8.5
Do.	80	hard	12	25	15	15-60	1.0-2.0	2.5-5.7
Do.	25	hard	17	25	30	15-60	0.6-1.0	1.0-4.2
Brown trout	18	hard	7	25	15	15-60	0.7-1.0	5.0-10.0
Do.	16	hard	12	25	15	15-60	0.9-1.2	3.5-8.5
Do.	16	hard	17	25	15	15-60	0.5-0.9	3.4-6.0
Brook trout	27	hard	7	25	15	15-60	1.0-1.6	3.0-8.0
Do.	27	hard	12	25	15	15-60	2.0-3.0	2.3-6.0
Do.	1.1	soft	12	25	5	15	1.1-1.2	2.2-3.0
Lake trout	25	hard	7	25	10	15-30	0.9-1.2	8.0-13.0
Do.	30	hard	12	25	15	15-60	0.8-1.5	3.5-8.0
Do.	25	hard	17	25	10	15-30	0.7-1.2	2.2-5.1

The same concentrations of $QdSO_4$ which were effective in the laboratory (20 to 25 mg/1) were effective on larger trout and salmon in field tests (table 4). The large fish exhibited stronger reflex actions when touched or gently squeezed.

The warmwater species generally required higher concentrations of the anesthetic than did the salmonids. Northern pike, black bullheads, and channel catfish were the most resistant fishes requiring up to 65 mg/1 of $QdSO_4$ for rapid anesthetization (table 5). Bluegills were the most susceptible, being anesthetized by 15 to 25 mg/1. On an active ingredient basis our 9 to 15 mg/1 for bluegills and 15 mg/1 for largemouth bass compare very favorably with the 15 mg/1 found to be effective on both species by Schoettger and Julin (1969). The warmwater fish recovered after 1 to 22 minutes in fresh water.

The speed at which a given concentration of $QdSO_4$ induced anesthesia was somewhat related to the size of fish, with larger fish requiring slightly longer times to reach the same stage (table 6). The differences were minimal and would not usually justify the use of higher concentrations on larger fish. When the size of fish was constant, higher concentrations sometimes induced anesthesia more rapidly. However, reducing the time needed for anesthetization by 1 to 2 minutes does not generally warrant the use of greatly increased concentrations.

The water temperature, in most cases, did not appreciably affect the efficacy of the anesthetic. Among salmonids, a single concentration was usually effective on any particular species at all temperatures. Northern pike and black bullheads were anesthetized by somewhat lower concentrations at temperatures of 22° to 27° C. than at 12° C.

TABLE 4.--Efficacy of quinaldine sulfate as an anesthetic for adult fish at field installations

Species and location	No. of fish	Average weight of fish (kg)	Effective concentration (mg/1)	Time in minutes to	
				Loss of equilibrium Stage 2	Recovery in fresh water
Coho salmon Platte River SFH[1], Michigan	3	3.5	25	3.3	3.5
Rainbow trout Manchester NFH[2], Iowa	12	0.3	25	0.9-1.5	3.0-4.0
Brown trout Manchester NFH, Iowa	21	1.1	25	0.8-1.2	5.3-8.3
Brook trout Osceola SFH, Wisconsin	31	0.7	25	0.9-2.3	2.0-4.5
Lake trout Crystal Springs SFH, Minnesota	6	1.5	25	2.5-3.0	---
Northern pike Lansing SFH, Iowa	9	1.6	30	6.0-11.5	9.8-22.0
Walleye Lansing SFH, Iowa	3	0.8	20	2.1-3.2	3.0-18.8

[1] State fish hatchery.
[2] National fish hatchery.

TABLE 5.--Efficacy of quinaldine sulfate as an anesthetic for warmwater fishes in laboratory tests

Species	Mean weight (grams)	Type of water	Temp. (°C.)	Concentration (mg/l)	Number of fish	Exposure time (min.)	Time in minutes to	
							Loss of equilibrium Stage 2	Recovery
Northern pike	115	soft	7	40	5	15	3.3-4.3	10.0-11.7
Do.	115	hard	12	40	5	15	2.5-4.2	4.0-6.1
Do.	115	soft	17	40	5	15	1.3-2.6	2.0-3.0
Do.	115	soft	22	25	5	15	2.5-2.9	0.9-1.1
Carp	60	hard	12	35	15	15-60	2.3-2.9	6.3-15.9
Do.	387	hard	22	25	10	5.5-15	1.7-4.5	2.5-5.0
Do.	387	hard	27	25	5	15	3.6-3.8	1.5-6.2
White amur	227	soft[1]	19	30	3	30	1.1-1.6	4.0-6.0
Black bullhead	208	hard	12	50	10	5.5-15	2.5-3.6	4.0-22.0
Do.	129	hard	22	25	10	5.5-15	2.8-3.7	1.7-4.3
Do.	129	hard	27	25	5	15	3.7	2.0-3.0
Channel catfish	1.8	hard	12	60	10	5.5-15	1.3-3.6	3.0-5.5
Do.	1.5	hard	17	60	5	15	0.9-1.1	1.8-6.2
Do.	1316	soft[1]	19	65	20	30	2.0-3.0	3.0-8.0
Bluegill	77	hard	17	15	5	15	1.7-2.1	2.0-3.7
Do.	80	hard	27	25	10	5.5-15	0.7-1.2	2.0-2.5
Do.	142	soft[1]	19	20	43	30	2.0-3.0	1.0-3.0
Largemouth bass	12	hard	17	25	5	15	1.1-1.5	2.8-3.5
Do.	908	soft[1]	19	30	30	30	1.8-3.0	3.0-10.0

[1] Spring water - Warm Springs Laboratory.

TABLE 6.--Examples of differences in rate of anesthesia among different size fish exposed to quinaldine sulfate in well water

Species	Mean weight (grams)	Temp. (°C.)	Concentration (mg/l)	Time to loss of equilibrium, Stage 2 (min.)
Rainbow trout	450	12	25	0.8-1.5
Do.	1.5	12	25	0.5
Do.	450	17	25	0.8-1.3
Do.	25	17	25	0.6-0.9
Lake trout	25	17	25	0.7-1.2
Do.	1.5	17	25	0.5-0.6

The hardness and pH of the water have a pronounced effect on the efficacy of $QdSO_4$. In soft, poorly-buffered water, the addition of 60 mg/l of $QdSO_4$ reduces the pH to 6.04 (Marking and Dawson, 1973). At that pH, a significant portion of the quinaldine ionizes and becomes unavailable to the fish (Schoettger and Julin, 1969; Sills and Allen, 1971). The relationship of hardness and pH to efficacy was demonstrated by comparing the efficacy on northern pike in two different waters at 12^{o} C. Forty mg/l were effective in hard water and 60 mg/l were ineffective in soft water.

DISCUSSION

Quinaldine sulfate proved to be an effective anesthetic for all of the species of fish on which it was tested. The fish were anesthetized to loss of equilibrium, Stage 2, in about 4 minutes or less, and all species but channel catfish survived 60 minutes of exposure to effective concentrations. A rather narrow range of concentrations (20 to 35 mg/l) was effective on salmonids, cyprinids, and centrarchids. Higher concentrations (40 to 65 mg/l) were required for northern pike and the ictalurids. The anesthetic is effective over a wide range of temperatures (7^{o} to 27^{o} C.) and in soft and hard waters. The species of fish used in the tests of $QdSO_4$ represent a wide range of physiological characteristics. Thus, the concentrations of 20 to 65 mg/l and exposures of up to 1 hour should serve as guidelines for use on most species of fish.

The only limiting factor on the efficacy of $QdSO_4$ is a pH below 6, at which the compound ionizes and becomes ineffective. The anesthetic itself is acidic and concentrations of 20 mg/l in very soft water (total hardness of 10 to 13 mg/l) and 60 mg/l in soft water (total hardness of 40 to 48 mg/l) lower the pH to near or below that point (Marking and Dawson, 1973). If the pH of the anesthetic solution is excessively low, it should be buffered back to the range of 6.5 to 7.0 with $NaHCO_3$.

Schoettger and Julin (1969) stated that 1 ml of quinaldine would anesthetize 94.5 kg of rainbow trout before the time to anesthetization increased. The effective concentrations of $QdSO_4$ were comparable, on an active ingredient basis, to those of practical grade quinaldine given by Schoettger and Julin (1969). By inference then, the weight of fish which can be anesthetized per unit of active ingredient of quinaldine and $QdSO_4$ are likely to be similar.

The major assets of $QdSO_4$, like those of quinaldine, are rapid anesthetization and a long safe-exposure time. In addition, $QdSO_4$ is more convenient to use than quinaldine because it does not have to be put into solution with an organic solvent. As with quinaldine, $QdSO_4$ does not completely inhibit reflex response. The reflex movements of large fish may be objectionable to some workers. A given degree of reflex response, however, may be a hindrance to one person and of no consequence to another.

CONCLUSIONS

1. Quinaldine sulfate is an effective anesthetic for most species of freshwater fish in concentrations of 20 to 65 mg/l.

2. The crystalline, water soluble material is convenient to handle and is as effective as practical grade quinaldine, on an active ingredient basis.

3. The efficacy of $QdSO_4$ is not greatly influenced by water temperature or size of fish.

4. In soft waters, high concentrations of $QdSO_4$ lower the pH of the solution, rendering the anesthetic ineffective.

5. The reflex action retained by fish under anesthesia may interfere with the handling of large fish.

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50. Residue of Quinaldine in Ten Species of Fish Following Anesthesia with Quinaldine Sulfate

By Joe B. Sills, John L. Allen, Paul D. Harman,
and Charles W. Luhning

United States Department of the Interior, Rogers C. B. Morton, *Secretary*
Nathaniel P. Reed, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife, Spencer H. Smith, *Director*
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RESIDUE OF QUINALDINE IN TEN SPECIES OF FISH FOLLOWING ANESTHESIA WITH QUINALDINE SULFATE

By Joe B. Sills, John L. Allen, Paul D. Harman,
and Charles W. Luhning
Fish Control Laboratories, La Crosse, Wisconsin
and Warm Springs, Georgia

ABSTRACT.--The concentration and persistence of residues of the anesthetic quinaldine in five species of both coldwater and warmwater fishes were measured following treatment with the new formulation quinaldine sulfate. Quinaldine accumulated in relation to increasing temperature, treatment concentration, and length of exposure. Mean concentrations of quinaldine residues ranged from 1.18 to 8.31 μg/g for the 0-hour withdrawal time. Initial residues were higher in warmwater species than in salmonids. Although a wide range of residue concentrations (0.80-9.34 μg/g) occurred immediately after exposure, these residues are dissipated rapidly when the fish are placed in fresh water. All fish tested after 24 hours of withdrawal in fresh water contained 0.01 μg/g or less of quinaldine residue with the exception of the rainbow trout treated at 7° C.

INTRODUCTION

Quinaldine sulfate was prepared by Allen and Sills (1973) as an improved form of the fish anesthetic quinaldine. In order to register this formulation, the concentration and persistence of residues in fish tissues must be known. Sills and Harman (1970) determined residue levels in striped bass (Morone saxatilis) following anesthesia with the quinaldine salt. In the present study, 10 species of coldwater and warmwater fishes (table 1) were anesthetized with quinaldine sulfate at various concentrations, temperatures, and times, and muscle tissue was analyzed for quinaldine residue.

TABLE 1.--Species of fish analyzed for quinaldine residues following anesthesia with quinaldine sulfate

Common Name	Scientific Name
Coho salmon	Oncorhynchus kisutch
Brown trout	Salmo trutta
Rainbow trout	Salmo gairdneri
Lake trout	Salvelinus namaycush
Brook trout	Salvelinus fontinalis
Northern pike	Esox lucius
Channel catfish	Ictalurus punctatus
Largemouth bass	Micropterus salmoides
Bluegill	Lepomis macrochirus
Walleye	Stizostedion vitreum

METHODS AND MATERIALS

Ten species of fish used in this study were treated with efficacious concentrations of quinaldine sulfate by Gilderhus et al. (1973). Temperatures of treatment ranged from 7° to 19° C., and exposure times ranged from 5.5 to 30 minutes. A wide range of concentrations (20 to 65 mg/l) was necessary because of the variety of species and temperatures involved.

Withdrawal times began when exposed fish were placed in fresh, flowing water for recovery. Three fish were used for residue analysis at 0, 1, 2, 4, either 6 or 8, and 24 hours. Samples of muscle tissue were obtained from homogenized fillets (Luhning and Harman, 1971) and were analyzed for quinaldine by gas chromatography using the methods of Allen and Sills (1970a and 1970b). Three samples were analyzed at each withdrawal. Quinaldine residues were measured to 0.01 μg/g, and any value less than this was considered zero.

RESULTS

Coho salmon

Coho salmon were the largest fish tested (table 2). They were treated with 25 mg/l of quinaldine sulfate at 4° C. Mean quinaldine residues for the 0-hour withdrawal interval were 1.18 and 1.78 μg/g for the 5.5- and 15-minute exposures, respectively. Quinaldine residue was less than 0.01 μg/g for both exposures after 24 hours of withdrawal from the anesthetic.

TABLE 2.--Residues of quinaldine in coho salmon muscle following anesthesia with 25 mg/l of quinaldine sulfate at 4° C.

Withdrawal interval	Exposure time (minutes)	Mean weight (kg)	Residues (μg/g)[1] Quinaldine	
			Mean	Range
control	0	2.03	0.00[2]	0.00
0-hour	5.5	4.07	1.18	1.03-1.26
1-hour	5.5	2.79	0.09	0.07-0.14
2-hour	5.5	3.83	0.03	0.01-0.06
4-hour	5.5	3.86	0.01	0.00-0.01
8-hour	5.5	3.25	0.00	0.00
24-hour	5.5	3.77	0.00	0.00
0-hour	15	3.96	1.78	1.03-2.46
8-hour	15	3.63	0.00	0.00
24-hour	15	2.69	0.00	0.00

[1] Each mean value represents the average of three analyses.
[2] Values less than 0.01 μg/g are reported as 0.00.

Brown trout

Brown trout were exposed to 25 mg/l of quinaldine sulfate at 12° C. for both 5.5 and 15 minutes. Quinaldine residues ranged from a mean of 1.44 μg/g after 5.5 minutes of exposure to 1.67 μg/g after 15 minutes of exposure. No detectable residue (less than 0.01 μg/g) remained after 8 hours of withdrawal from the anesthetic (table 3).

TABLE 3.--Residues of quinaldine in muscle of brown trout after anesthesia with 25 mg/l of quinaldine sulfate at 12° C.

Withdrawal interval	Exposure time (minutes)	Mean weight (grams)	Residues (μg/g)[1] Quinaldine	
			Mean	Range
control	0	582	0.00[2]	0.00
0-hour	15	646	1.67	1.32-2.14
1-hour	15	525	0.65	0.48-0.86
2-hour	15	521	0.09	0.05-0.14
4-hour	15	584	0.02	0.02-0.02
8-hour	15	592	0.00	0.00
24-hour	15	515	0.00	0.00
0-hour	5.5	542	1.44	1.21-1.81
2-hour	5.5	555	0.03	0.02-0.04
8-hour	5.5	492	0.00	0.00

[1] Each mean value represents the average of three analyses.
[2] Values less than 0.01 μg/g are reported as 0.00.

Rainbow trout

Rainbow trout were tested most extensively. Fish weighing from approximately 100 to 550 grams which had been exposed to 25 mg/l of quinaldine sulfate were analyzed for quinaldine residue. At 12° C., fish exposures to the anesthetic were 5.5 and 15 minutes. At 7° and 17° C. the fish were exposed to the anesthetic for 15 minutes (table 4). A quinaldine residue was detectable immediately following exposure to quinaldine sulfate and the initial residue was higher as the temperature and exposure time increased.

TABLE 4.--Residues of quinaldine in muscle of rainbow trout anesthetized with 25 mg/l of quinaldine sulfate at three temperatures

Withdrawal interval	Treatment		Mean weight (grams)	Residues (μg/g)[1] Quinaldine	
	Exposures (minutes)	Temp. (°C.)		Mean	Range
control	0	7	384	[2] 0.00	0.00
0-hour	15	7	419	3.53	2.40-4.80
1-hour	15	7	335	1.99	1.20-3.22
2-hour	15	7	480	0.67	0.64-0.72
4-hour	15	7	452	0.64	0.46-0.75
24-hour	15	7	547	0.17	0.15-0.18
control	0	12	135	0.00	0.00
0-hour	15	12	452	2.62	0.80-5.33
1-hour	15	12	460	0.73	0.46-1.28
2-hour	15	12	357	0.22	0.16-0.26
4-hour	15	12	327	0.04	0.01-0.06
8-hour	15	12	403	0.00	0.00
24-hour	15	12	455	0.00	0.00
0-hour	5.5	12	142	1.80	1.10-2.40
1-hour	5.5	12	131	0.32	0.30-0.33
2-hour	5.5	12	145	0.10	0.08-0.11
4-hour	5.5	12	118	0.03	0.03-0.03
8-hour	5.5	12	93	0.01	0.01-0.01
24-hour	5.5	12	111	0.00	0.00
control	0	17	135	0.00	0.00
0-hour	15	17	392	4.51	2.08-6.25
1-hour	15	17	433	2.02	1.55-2.87
2-hour	15	17	440	1.44	0.77-2.17
4-hour	15	17	360	1.11	0.90-1.33
8-hour	15	17	466	0.44	0.38-0.54

[1] Each mean value represents the average of three analyses.
[2] Values less than 0.01 μg/g are reported as 0.00.

The rainbow trout exposed to the anesthetic for 15 minutes at 7° C. contained a mean quinaldine residue of 3.53 μg/g at the 0-hour withdrawal and 0.17 μg/g after 24 hours of withdrawal. Those treated at 12° C. for 5.5 and 15 minutes contained a mean residue of quinaldine of 1.80 and 2.62 μg/g, respectively. After 24 hours of withdrawal at 12° C., the quinaldine residue concentrations had fallen below 0.01 μg/g in both the 5.5- and 15-minute exposures. The rainbow trout treated at 17° C. contained a mean residue of 4.51 μg/g of quinaldine at 0-hour withdrawal and 0.44 μg/g quinaldine after 8 hours of withdrawal. A 24-hour withdrawal was not available for this set of samples.

Lake trout and brook trout

Lake trout exposed to the anesthetic at 12° C. for 5.5 and 15 minutes had quinaldine residues ranging from 1.49 to 3.90 μg/g at the 0-hour withdrawal, and were 0.01 μg/g or less after 8 hours of withdrawal (table 5). Brook trout were treated for 5.5 minutes at 9° C. and contained a mean quinaldine residue of 3.13 μg/g at the 0-hour withdrawal and 0.01 μg/g after 8 hours of withdrawal. No quinaldine residue was detected in any of these fish after 24 hours of withdrawal.

Northern pike

Northern pike treated with 30 mg/l of quinaldine sulfate for 30 minutes at 7° and 12° C. were analyzed for quinaldine residue (table 6). Those treated at 7° C. contained a mean quinaldine residue of 4.80 μg/g at the 0-hour withdrawal and less than 0.01 μg/g of quinaldine residue after 24 hours of withdrawal. Those treated at 12° C. contained a mean quinaldine residue of 4.40 μg/g at the 0-hour withdrawal and 0.17 μg/g after 4 hours of withdrawal. No samples were available for the 12° C. treatment with longer than 4 hours of withdrawal.

Channel catfish, largemouth bass and bluegill

The warmwater species including channel catfish, largemouth bass, and bluegill were exposed to the highest concentrations of the anesthetic and at the highest temperature, 19° C., for 30 minutes (table 7). This resulted in the highest quinaldine residues encountered

TABLE 5.--Residues of quinaldine in muscle of lake trout and brook trout after anesthesia with 25 mg/l of quinaldine sulfate

Withdrawal interval	Treatment		Mean weight (grams)	Residues (μg/g)[1] Quinaldine	
	Exposure (minutes)	Temp. (°C.)		Mean	Range
Lake trout					
control	0	12	1630	[2]0.00	0.00
0-hour	5.5	12	1580	2.04	1.49-2.59
1-hour	5.5	12	1270	0.17	0.10-0.28
2-hour	5.5	12	1440	0.11	0.08-0.15
4-hour	5.5	12	1690	0.02	0.01-0.03
8-hour	5.5	12	1500	0.01	0.01-0.01
24-hour	5.5	12	1170	0.00	0.00
0-hour	15	12	1430	3.50	3.09-3.90
8-hour	15	12	1620	0.01	0.01-0.02
24-hour	15	12	1470	0.00	0.00
Brook trout					
control	0	9	300	0.00	0.00
0-hour	5.5	9	302	3.13	2.27-3.41
1-hour	5.5	9	344	0.27	0.22-0.30
2-hour	5.5	9	332	0.09	0.06-0.14
4-hour	5.5	9	297	0.03	0.02-0.04
8-hour	5.5	9	370	0.01	0.00-0.01
24-hour	5.5	9	307	0.00	0.00

[1] Each mean value represents the average of three analyses.
[2] Vales less than 0.01 μg/g are reported as 0.00.

at the 0-hour withdrawal, but after 24 hours of withdrawal they were 0.01 μg/g or less.

Walleye

Walleye treated with 20 mg/l of quinaldine sulfate for 30 minutes at 7° C. contained a mean quinaldine residue of 2.60 μg/g at the 0-hour withdrawal and 0.04 μg/g after 6 hours of withdrawal (table 8). No samples were available with longer than 6 hours of withdrawal.

TABLE 6.--Residues of quinaldine in muscle tissue of northern pike following anesthesia with 30 mg/l of quinaldine sulfate

Withdrawal interval	Treatment		Mean weight (kg)	Residues (μg/g)[1] Quinaldine	
	Exposure (minutes)	Temp. (°C.)		Mean	Range
control	0	7	1.43	[2]0.00	0.00
0-hour	30	7	1.89	4.80	2.10-8.50
1-hour	30	7	1.18	1.10	1.00-1.20
2-hour	30	7	1.00	0.51	0.33-0.63
4-hour	30	7	1.06	0.06	0.05-0.08
24-hour	30	7	1.12	0.00	0.00
control	0	12	1.48	0.00	0.00
0-hour	30	12	1.91	4.40	2.80-5.40
2-hour	30	12	1.63	0.68	0.61-0.76
4-hour	30	12	1.43	0.17	0.05-0.42

[1] Each mean value represents the average of three analyses.
[2] Values less than 0.01 μg/g are reported as 0.00.

TABLE 7.--Residues of quinaldine in muscle of channel catfish, bluegill, and largemouth bass anesthetized with quinaldine sulfate for 30 minutes at 19° C.

Withdrawal interval	Treatment $QdSO_4$ (mg/l)	Mean weight (grams)	Residues (μg/g)[1] Quinaldine	
			Mean	Range
Channel catfish				
control	0	1,270	[2]0.00	0.00
0-hour	65	1,320	8.31	7.79-9.34
1-hour	65	1,680	5.55	5.44-5.86
2-hour	65	680	1.68	1.60-1.76
4-hour	65	1,320	0.91	0.82-1.00
6-hour	65	1,090	0.26	0.15-0.40
24-hour	65	1,680	0.01	0.01-0.01

See footnotes at end of table.

TABLE 7.--Residues of quinaldine in muscle of channel catfish, bluegill, and largemouth bass anesthetized with quinaldine sulfate for 30 minutes at 19° C.--Continued.

Withdrawal interval	Treatment $QdSO_4$ (mg/l)	Mean weight (grams)	Residues ($\mu g/g$)[1] Quinaldine	
			Mean	Range
Bluegill				
control	0	115	0.00	0.00
0-hour	20	152	3.72	3.43-4.07
1-hour	20	180	0.52	0.46-0.63
2-hour	20	135	0.33	0.12-0.61
4-hour	20	165	0.14	0.03-0.22
6-hour	20	110	0.03	0.02-0.04
24-hour	20	112	0.00	0.00-0.01
Largemouth bass				
control	0	1,090	0.00	0.00
0-hour	30	1,000	6.07	5.04-7.20
1-hour	30	1,000	0.76	0.44-1.00
2-hour	30	1,040	0.20	0.17-0.21
4-hour	30	590	0.05	0.04-0.06
6-hour	30	730	0.05	0.03-0.06
24-hour	30	770	0.00	0.00

[1] Each mean value represents the average of three analyses.
[2] Values less than 0.01 $\mu g/g$ are reported as 0.00.

TABLE 8.--Residues of quinaldine in muscle of walleye following anesthesia with 20 mg/l of quinaldine sulfate at 7° C.

Withdrawal interval	Treatment Exposure (minutes)	Mean weight (kg)	Residues ($\mu g/g$)[1] Quinaldine	
			Mean	Range
Walleye				
control	0	0.81	0.00	0.00
0-hour	30	1.32	2.60	2.20-3.00
1-hour	30	1.92	1.30	1.10-1.60
2-hour	30	0.82	0.93	0.70-1.20
6-hour	30	0.82	0.04	0.02-0.07

[1] Each mean value represents the average of three analyses.
[2] Values less than 0.01 $\mu g/g$ are reported as 0.00.

DISCUSSION

Residues of quinaldine dissipated rapidly from the muscle of all fish in this study. A representative curve for disappearance of quinaldine residue is shown in figure 1. The curves represent the averages of mean values obtained on coho salmon, rainbow trout, brown trout, brook trout, and lake trout exposed to 25 mg/l of quinaldine sulfate for 15 minutes at 12° C., and channel catfish, largemouth bass, and bluegill exposed to 65, 30, and 20 mg/l of quinaldine sulfate, respectively, for a period of 30 minutes at 19° C. The regression of the residues in both groups of fish is similar to that of MS-222 reported by Walker and Schoettger (1967).

The warmwater fish accumulated higher concentrations of residues than the salmonids. This appears to be directly related to higher concentrations of anesthetic, higher temperature, and longer exposure time. Though there was wide variation in residue concentrations at the 0-hour withdrawal time, residues in all fish, except rainbow trout treated at 7° C., were at undetectable levels within 24 hours. The rainbow trout treated at 7° C., the coldest temperature tested, contained a mean quinaldine residue of 3.53 $\mu g/g$ at the 0-hour withdrawal and after 24 hours of withdrawal from the anesthetic had fallen to a mean of 0.17 $\mu g/g$.

CONCLUSIONS

1. The residues of quinaldine in all the species tested varied considerably at the 0-hour withdrawal depending on the concentration of anesthetic used, temperature, and exposure time. An increase in any of these parameters also increased residue concentration at the 0-hour withdrawal time.
2. The residue concentration fell below 0.01 $\mu g/g$ after 24 hours of withdrawal in all cases, except the rainbow trout treated at 7° C.
3. The residues of quinaldine dissipated more slowly at the lower temperatures. After 24 hours of withdrawal at 7° C., the rainbow trout still contained an average of 0.17 $\mu g/g$ of quinaldine residue.

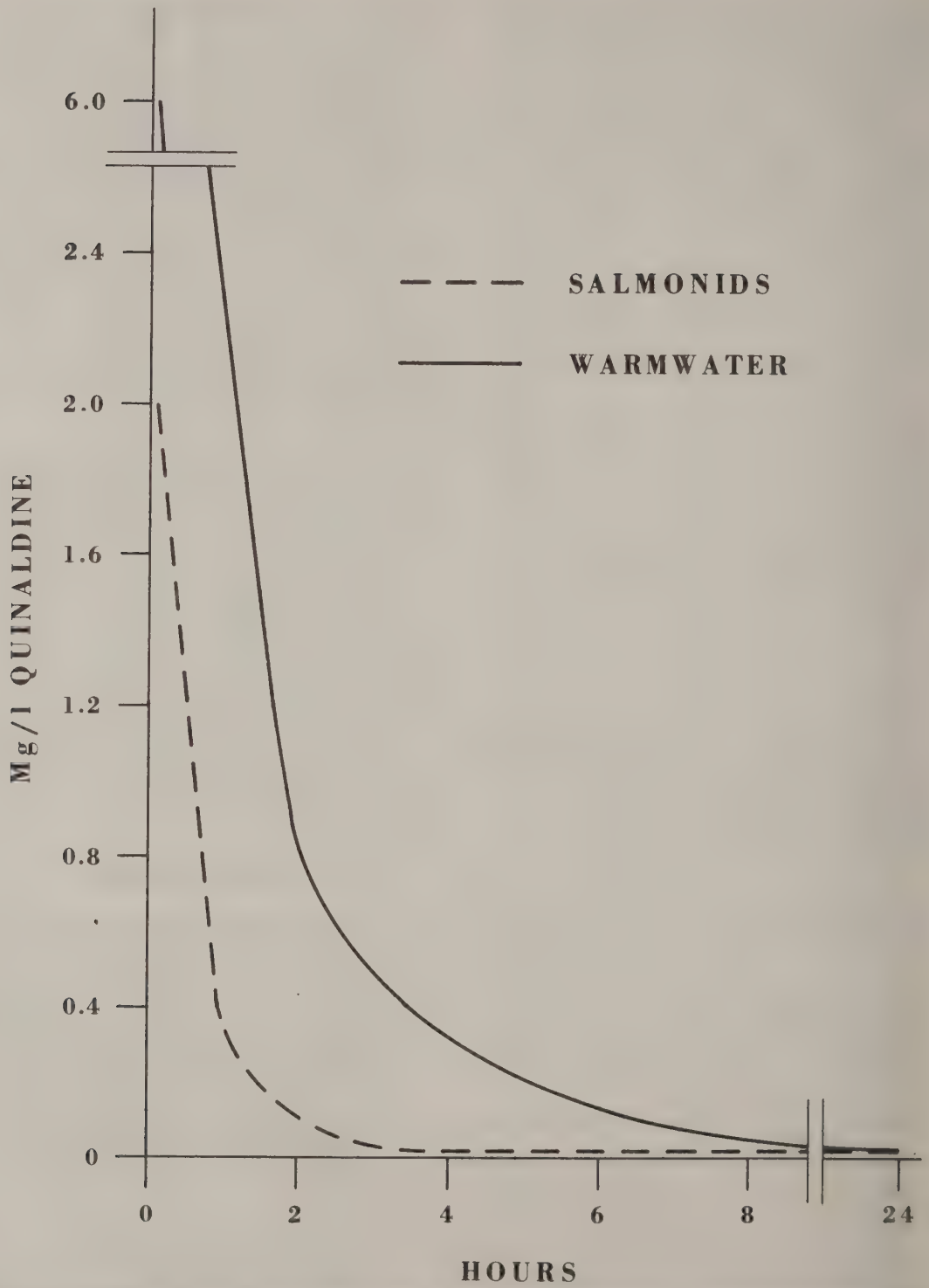

Figure 1.—Regression of quinaldine residues in muscle tissue of salmonids exposed to quinaldine sulfate at 12° C., and warmwater fish at 19° C. as a function of time.

4. Warmwater species had higher initial quinaldine residues than salmonids.

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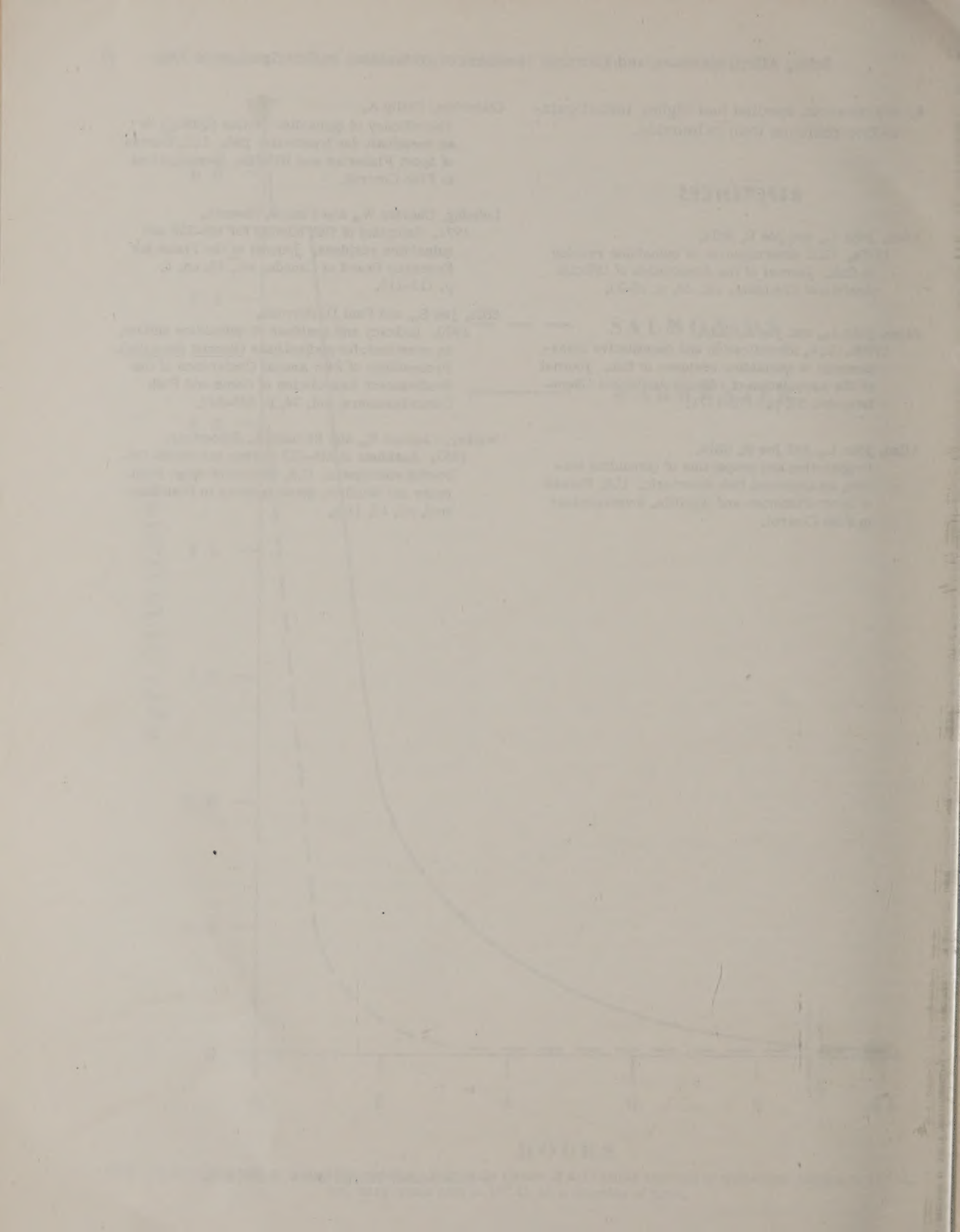

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41. Identification of MS-222 Residues in Selected Fish Tissues by Thin Layer Chromatography, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1970. 7 p.
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44. A Review of Literature on TFM (3-trifluormethyl-4-nitrophenol) as a Lamprey Larvicide, by Rosalie A. Schnick. 1972. 31 p.

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45. Residues of MS-222 in Northern Pike, Muskellunge, and Walleye, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1972. 8 p.
46. Methods of Estimating the Half-Life of Biological Activity of Toxic Chemicals in Water, by Leif L. Marking. 1972. 9 p.

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The Department works to assure the wisest choice in managing all our resources so that each shall make its full contribution to a better United States now and in the future.

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